CLAIMS

What is claimed is:

1. An isolated single-chain polypeptide comprising:
   a) a first amino acid sequence region comprising
      i) a first domain comprising a binding element able to specifically bind a target cell surface marker under physiological conditions; and
      ii) a second domain comprising a translocation element able to facilitate the transfer of a polypeptide across a vesicular membrane; and
   b) a second amino acid sequence region comprising a therapeutic element having biological activity when released into the cytoplasm of the target cell,

   wherein said first and second amino acid sequence regions are separated by a third amino acid sequence region comprising a protease cleavage site which is cleaved when exposed to a protease, provided that said protease is not normally expressed by a cell expressing said single-chain polypeptide.

2. The polypeptide of claim 1 wherein said first amino acid sequence region comprises at least a portion of a clostridial neurotoxin H chain.

3. The polypeptide of claim 2 wherein said portion of the clostridial neurotoxin H chain is derived from the neurotoxin heavy chain of *C. botulinum* subtype A.

4. The polypeptide of claim 2 wherein said second amino acid sequence region comprises at least a portion of a clostridial neurotoxin L chain.

5. The polypeptide of claim 4 wherein said portion of the clostridial neurotoxin L chain is derived from that obtained from one of *C. botulinum* subtype A, B or E.

6. The polypeptide of claim 2 wherein said portion of the clostridial neurotoxin H chain is derived from that obtained from *C. tetani.*

7. The polypeptide of claim 2 wherein said portion of the clostridial neurotoxin H chain is derived from the neurotoxin heavy chain of an organism selected from the group consisting of *C. botulinum* subtype B, *C. botulinum* subtype C; *C. botulinum* subtype D; *C. botulinum* subtype E; *C. botulinum* subtype F; *C. botulinum* subtype G; *C. baratii;* and *C. butyricum.*

8. The polypeptide of claim 1 wherein said first domain comprises a binding element able to bind the surface of pancreatic acinar cells.

9. The polypeptide of claim 8 wherein said second domain comprises a clostridial neurotoxin L chain.

10. The polypeptide of claim 1 wherein said binding element specifically binds a target cell surface marker of a sensory afferent neuron.

11. The polypeptide of any one of claims 1-10 wherein said protease cleavage site is cleaved by a protease selected from the group consisting of:

a) A non-human enterokinase;

b) tobacco etch virus protease;

c) a protease derived from *Bacillus subtilus*;

d) a protease derived from *Bacillus amyliquifaciens*;

e) a protease derived from a rhinovirus;

f) papain;

g) an insect papain homolog and

h) a crustacian papain homolog.

12. The polypeptide of claim 1 wherein said protease cleavage site comprises an amino acid sequence selected from the group consisting of:

a) DDDDK;

b) EXXYXQS/G;

c) HY;

d) YH; and

e) LEVLFQGP,

wherein X = any amino acid.

13. The polypeptide of claim 1 or claim 11 further comprising a binding tag.

14. The polypeptide of claim 13 wherein said binding tag comprises a target-binding portion of a polypeptide selected from the group consisting of His_6, monoclonal antibodies, maltose binding protein, glutathione-S-transferase, protein A, and calmodulin binding protein.

15. The polypeptide of claim 1 wherein said first amino acid sequence region comprises at least a portion of a heavy chain derived from a first clostridial neurotoxin subtype and said second amino acid sequence region comprises at least a portion of a light chain derived from a second clostridial neurotoxin subtype, wherein said first and second clostridial neurotoxin subtypes are not the same.

16. The polypeptide of claim 15 wherein said heavy chain portion is from a heavy chain selected from the group consisting of HC_A, HC_B, HC_{C1}, HC_D, HC_E, HC_F, and HC_G and said light chain portion is from a light chain selected from the group consisting of LC_A, LC_B, LC_{C1}, LC_D, LC_E, LC_F, LC_G , and wherein said heavy and light chain portions hare from different clostridial neurotoxin subtypes.

17. The polypeptide of claim 16 wherein at least one of said heavy and light chain portions comprise less than the entire heavy or light chain from said clostridial neurotoxin subtype, respectively.

18. The polypeptide of claim 16 wherein at least one of said heavy and light chain portions comprises the entire heavy or light chain from said clostridial neurotoxin subtype, respectively.

19. The polypeptide of any of claims 1, 2, 8, 10 or 15 wherein said first or second amino acid sequence region is modified to eliminate at least one amino acid sequence specifically cleaved by a protease.

20. A plasmid having a nucleic acid sequence region comprising an open reading frame encoding a cleavable single-chain polypeptide, said open reading frame comprising:

a) a first nucleotide sequence region comprising

i) a first portion encoding a first amino acid sequence region comprising a binding element able to specifically bind a target cell surface marker under physiological conditions; and

iii) a second portion encoding a second amino acid sequence region comprising a translocation element able to facilitate the transfer of a polypeptide across a vesicular membrane;

b) a second nucleotide sequence region encoding a third amino acid sequence region comprising a therapeutic element having biological activity when released into the cytoplasm of the target cell, and

wherein said first and second nucleotide sequence regions are separated by a third nucleotide sequence region encoding a fourth amino acid sequence comprising a protease cleavage site which is cleaved when exposed to a protease, provided said protease is not normally expressed by a cell expressing said single-chain polypeptide, and wherein said single-chain polypeptide is expressed by said plasmid within a suitable host cell.

21. The plasmid of claim 20 wherein said first or second nucleotide sequence region further encodes an amino acid sequence region comprising a binding tag.

22. The plasmid of claim 21 wherein said binding tag comprises a target-binding portion of a polypeptide selected from the group consisting of

His_6, monoclonal antibodies, maltose binding protein, glutathione-S-transferase, protein A, and calmodulin binding protein.

23. The plasmid of claim 20 wherein said first nucleotide sequence region encodes at least a portion of a clostridial neurotoxin heavy chain.

24. The plasmid of claim 23 wherein said first nucleotide sequence region encodes at least a portion of a *Clostridium botulinum* neurotoxin heavy chain.

25. The plasmid of claim 23 wherein said first nucleotide sequence region encodes at least a portion of a *Clostridium tetani* neurotoxin heavy chain.

26. The plasmid of either of claims 20 or 23 wherein said second nucleotide sequence region encodes at least a portion of a clostridial neurotoxin light chain.

27. The plasmid of claim 26 wherein said second nucleotide sequence region encodes at least a portion of a *Clostridium botulinum* neurotoxin light chain.

28. The plasmid of claim 26 wherein said second nucleotide sequence region encodes at least a portion of a *Clostridium tetani* neurotoxin light chain.

29. The plasmid of claim 20 wherein said first nucleotide sequence region encodes a binding element which will specifically bind a cell type other than a motor neuron.

30. The plasmid of claim 29 wherein said first nucleotide sequence region encodes a binding element that will specifically bind a cell type selected from the group consisting of a pancreatic acinar cell and a sensory afferent neuron.

31. The plasmid of claim 20 wherein said second nucleotide sequence region encodes a therapeutic element other than a clostridial neurotoxin light chain.

32. A method of making a single-chain polypeptide derived from a clostridial neurotoxin comprising:

a) inserting the plasmid of any one of claims 20-31 into a suitable host cell,

b) growing said host cell in culture, and

c) permitting or inducing the host cell to express the single chain polypeptide encoded by said plasmid.

33. A method of purifying the recombinant single chain polypeptide of claim 13 comprising the steps: lysing a cell expressing said single chain polypeptide to produce a cell lysate, and contacting said cell lysate with a target compound so as to form a specific binding complex capable of

being immobilized comprising said binding tag and said target compound.

34. An isolated single-chain polypeptide comprising:

a) a first amino acid sequence region comprising

i) a first domain comprising a binding element able to specifically bind a target cell surface marker under physiological conditions; and

ii) a second domain comprising a translocation element able to facilitate the transfer of a polypeptide across a vesicular membrane; and

b) a second amino acid sequence region comprising a therapeutic element having biological activity when released into the cytoplasm of the target cell,

wherein said first and second amino acid sequence regions are separated by a third amino acid sequence region comprising the interchain loop region of a BoNT/E neurotoxin.